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PHOSPHOCHOLINETRANSFERASE ACTIVITY IN PLASMA MEMBRANE: EFFECT OF DIET

K.M. Hargreaves and M.T. Clandinin

Departments of Medicine and Foods & Nutrition,
University of Alberta, Edmonton, Canada

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The presence of phosphocholinetransferase, a component of the CDP-choline pathway for phosphatidylcholine biosynthesis is demonstrated in brain synaptic plasma membrane. Activity of phosphocholinetransferase is higher in weanling versus adult tissues, and responds to alterations in fat intake. The implications of phosphocholinetransferase activity in plasma membrane, and dietary manipulation of phosphatidylcholine biosynthesis via this pathway are discussed.

The major route of membrane phosphatidylcholine biosynthesis is via the CDP-choline pathway, and was originally described for liver microsomes (1). The membrane bound phosphocholinetransferase component of this pathway is also present in neuronal membranes of neonatal rabbits (2) and in myelin (3). Phosphocholinetransferase activity present in the plasma membrane fraction utilizes endogenous membrane diacylglycerols to produce phosphatidylcholine. Presence of this enzyme activity in plasma membrane implies that phosphatidylcholine may be synthesized 'in situ' rather than translocated from the endoplasmic reticulum to the plasma membrane.

Diet affects phosphatidylcholine content and composition in synaptic plasma membrane of rat brain (4). Phosphocholinetransferase activity was examined in membrane fractions to demonstrate whether or not phosphocholinetransferase activity is present in the plasma membrane, and if previous reports indicating an effect of diet on membrane phosphatidylcholine content can be attributed to diet-induced alterations in phosphatidylcholine production via the CDP-choline pathway.

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Materials and Methods

Animals and diets: Weanling male Sprague-Dawley rats were fed standard lab chow (Wayne Lab-Blox) or semi-purified diets containing 20% (w/w) fat (5), for 24 days. Fatty-acid composition of dietary fat mixtures are illustrated (Table 1). Cholesterol was added to fish oil and linseed oil diets at a level of 2% of the total diet. Rats were housed individually with diet and water supplied ad libitum. Growth rates were similar for animals fed all diet treatments. Three rat brains were pooled per sample for isolation of synaptosomal plasma membrane and microsomal fractions.

Membrane isolation: Synaptosomal and microsomal membranes were isolated from rat brain by preparative ultracentrifugation and membrane purity characterized enzymatically as described previously (5,6).

Phosphocholinetransferase assay: Assays were performed at physiological conditions (37°C, pH 7.4) at protein concentrations within the linear range of reaction rate. Membrane fractions were incubated for 10 min. in the presence of 50 mM Tris-HCl, 25 mM $MgCl_2$, 6 mM dithiothreitol and 0.14 mM [^{14}C]-CDP-choline (2500 dpm/nmol; NEN, Boston, Mass). Unlabelled CDP-choline was purchased from Sigma Chemical Co., St. Louis, MO. The reaction was stopped with 3 ml chloroform:methanol:2N HCl (2:1:0.1 v/v/v), lipid extracted, washed twice with methanol:water (1:1 v/v) + 0.1M KCl, dried under nitrogen, resuspended in hexane and counted in 5 ml of fluor (Aquasol, NEN, Boston, Mass.). Background correction was made from a control reaction mixture. Samples were counted in a Beckman LS-5801 liquid scintillation spectrometer (Beckman Instruments Inc., Irvine, CA). Counting efficiency was approximately 97%, and all counts were corrected for counting efficiency. Membrane protein was determined by a modified Lowry method (7).

Table 1
Fatty Acid Composition of Diets

DIET FATTY ACID (% w/w)	Chow	SFO[a]	SBO	SAF	TAL	LO	FO
C12:0				0.4			
C14:0				0.3	3.8	0.1	0.1
C16:0[b]	16.5	6.5	13.3	7.6	26.8	10.3	7.6
C16:1[b]		0.2			0.3	0.7	10.9
C17:0					1.7	0.3	2.0
C18:0[b]	3.4	3.8	4.1	2.5	46.5	6.3	6.2
C18:1[b]	18.0	17.0	23.6	10.3	5.1	24.6	10.6
C18:2(6)[c]	42.8	71.9	52.7	77.1	10.9	16.2	1.6
C18:3(3)	4.5	0.2	5.8	1.0	1.4	36.9	6.0
C20:0				0.3	0.5	-	-
C20:4(6)					0.2		1.2
C20:5(3)							27.5
C22:5(3)							2.2
C22:6(3)							8.8

Diets contained 20% (w/w) fat as described in Methods, except chow which contained 4% w/w fat.

[a]SFO - Sunflower oil; SBO - Soya bean oil; SAF - Safflower oil; TAL - Beef tallow; LO - Linseed oil; FO - Fish oil.

[b]Includes ω9, ω7 and ω5 isomers.

[c]Numbers in brackets represent the position of double-bonds in the fatty acids, from the methyl end.

Statistical Analysis: Differences between diet treatments in phosphocholinetransferase activity were examined by Student's two-tailed t-tests or by multivariate analysis of variance and Neuman-Keuls multiple range test.

Results and Discussion

It is evident that phosphocholinetransferase activity is present in purified plasma membrane fractions of brain (Table 2). This activity is not due to microsomal contamination of plasma membrane fractions based on assays of microsomal markers (e.g. absence of RNA). Data from brain indicates higher phosphocholinetransferase activity in weanling animals versus adult animals, and this elevated activity in young animals is reflected in both microsomal and plasma membrane fractions. Synaptic plasma membrane phosphocholinetransferase activity may have a particularly important contribution to phosphatidylcholine production during the rapid growth of weanling animals, particularly as the activity in synaptic plasma membrane in relation to microsomal membranes is highest in young animals (Table 2). In this regard, the specific activity of phosphocholinetransferase in developing rabbit cerebrum appears to correlate with morphological and compositional events in the developing brain in the last trimester of gestation and first 3 weeks of postnatal life (8).

The ability of plasma membrane to synthesize phospholipid, and therefore control plasma membrane physico-chemical environment and thus perhaps function, to some degree independently of the microsomal membrane fraction, is

Table 2
Phosphocholinetransferase Activity

	nmoles phosphatidylcholine synthesized/mg protein/ 10 min	
Brain:	Synaptic Plasma Membrane	Microsomal Membrane
Weanling	4.1 ± 1.4 (12)*	8.6 ± 1.0 (12)
Adult	0.36 ± 0.02 (24)	1.2 ± 0.04 (24)

Values are means ± S.D. *(n)

Adult animals were fed diets containing sunflower oil and soya bean oil, for 24 days.

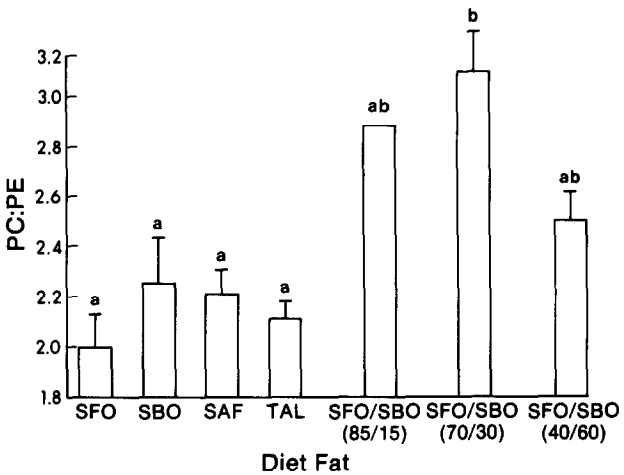

Figure 1. Values represent means ± S.E., n=4-11. Animals were fed semi-purified diets containing 20% (w/w) fat for 24 days, as described in Methods. Membrane phosphatidylcholine and phosphatidylethanolamine content was calculated from quantitative G.L.C. analysis of the fatty acid content of isolated phospholipid using C19:0 as an internal standard.

Abbreviations: SFO = Sunflower oil, SBO = Soya-bean oil, SAF = Safflower oil, TAL = Beef tallow; 85/15, 70/30 and 40/60 refer to % (w/w) of Sunflower and Soya-bean oil mixtures. PC = Phosphatidylcholine, PE = Phosphatidylethanolamine.

suggestive of a more important role for the plasma membrane than previously conceived. It also follows that the role of the plasma membrane may be greater during periods of rapid growth or when subjected to altered physiological conditions. One physiological condition which normally changes is diet. In agreement with previous results (4), diet affects synaptic plasma membrane phosphatidylcholine content (Figure 1). The route by which membrane phosphatidylcholine content is altered is likely complex, and may involve factors such as membrane cholesterol content, membrane phospholipid fatty-acid composition and many membrane-associated enzyme activities: acyltransferases, fatty acid elongation/desaturation, base-exchange, phospholipid synthesis via CDP-pathways or methyltransferase activity for phosphatidylcholine biosynthesis. By feeding diets of varying fatty acid composition, it is apparent that synaptic plasma membrane phosphocholinetransferase activity can be altered (Figure 2). Soya bean oil, safflower oil and beef tallow diets stimulate phosphocholinetransferase activity up to 2-fold over activity observed for control animals fed a diet containing sunflower oil. Animals fed

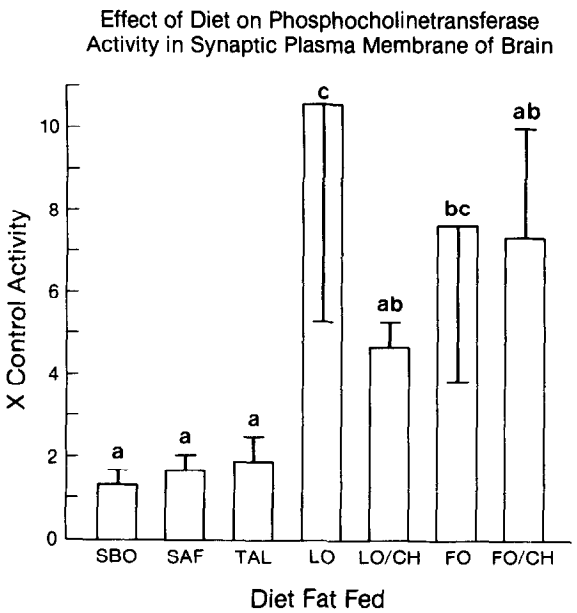

Figure 2. Values represent means ± S.D. for phosphocholinetransferase activity compared with activity from animals fed a diet containing sunflower oil: 0.34±0.03 nmoles/mg protein/10 min. Synaptic plasma membrane fractions were isolated as described in Methods. The diet treatments are as follows: SBO = soya bean oil; SAF = safflower oil; TAL = beef tallow; LO = linseed oil; LO/CH = linseed oil + 2% cholesterol; FO = fish oil; FO/CH = fish oil + 2% cholesterol (Table 1).

linseed oil show the highest levels of phosphocholinetransferase activity approaching the level of activity observed for weanling animals (Figure 2, Table 2). Animals fed fish oil also demonstrate high levels of phosphocholinetransferase activity. Addition of cholesterol to the linseed oil diet significantly depressed phosphocholinetransferase activity, but activity remains significantly higher than observed for control animals fed sunflower oil. Addition of cholesterol to the fish oil diet did not alter phosphocholinetransferase activity. Feeding young rats diets rich in cholesterol has been shown to increase liver phosphatidylcholine biosynthesis due to an increase in cytosolic phospholipid and an increase in translocation of cytidylyltransferase from the cytosol to the microsomes (9). Cytidylyltransferase activity was not measured in this study, but phosphocholinetransferase activity was depressed or remained constant when weanling rats were fed linseed oil or fish oil diets for 24 days. Cholesterol content in the membrane may be affecting phosphocholinetransferase conformation and activity.

Diet alters synaptic plasma membrane and microsomal membrane phosphatidylcholine content in a manner that correlates with membrane cholesterol content (4). Phosphatidylcholine content in these membrane fractions is also altered during growth (weanling vs. adult; 4). These changes in membrane composition may alter specific membrane-associated functions such as synaptic plasma membrane acetylcholinesterase activity (10), which are dependent on membrane lipid environment for activity. Control mechanisms regulating phosphatidylcholine synthesis via the CDP-choline pathway and the effect of diet on this control mechanism are not clear. Cell culture work has shown a stimulatory effect of fatty acids on the activity of cytidylyltransferase, the rate-limiting enzyme of the CDP-choline pathway (11,12). It is conceivable that diet may alter the availability of free fatty acids in the cytosol, and therefore translocation of cytidylyltransferase to the membrane. It is also conceivable that diet may affect the nature or pool of diglycerides in the membrane, but there does not appear to be specificity towards membrane diglyceride composition (13,14), and different phosphatidylcholine species are more likely to be formed by remodeling via deacylation-reacylation reactions (15). Choy et al. suggest that cytosolic diglycerides are the aggregating factor required to form active cytidylyltransferase (16). Several studies suggest that the stimulatory role of fatty-acids is a result of an altered phosphocholinetransferase conformation, which makes specific diglyceride species more acceptable substrates (17,18). The results of these experiments are based on providing a supply of exogenous diglyceride species as substrate and may not directly apply to the in vivo situation. The present experiments demonstrate activity utilizing endogenous membrane diglyceride. Diet induces change in membrane phospholipid composition (4,5). Phosphocholinetransferase activity may therefore be directly affected by associated transitions in protein-lipid interactions. Further research is needed to assess mechanisms for stimulation of phosphocholinetransferase activity. The significant diet-induced increase in synaptic plasma membrane phosphocholinetransferase activity (Figure 2) is

very exciting in light of the importance of phosphatidylcholine in the membrane, and changes in brain tissue known to occur in ageing or degenerative conditions such as Alzheimer's disease. The role of this pathway for phosphatidylcholine synthesis in the plasma membrane may be particularly important during periods of growth and development or in altered physiologic states. One could postulate that diet may represent an effective therapeutic tool with which to normalize specific membrane-associated functions which depend, in part, upon the phosphatidylcholine content of the plasma membrane.

Acknowledgements

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